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3-Hydroxypropylmercapturic Acid: a Biologic Marker of Exposure to Allylic and Related Compounds

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3-Hydroxypropylmercapturic acid [3-OHPrMCA, *S*-(3-hydroxypropyl)-*N*-acetyl-L-cysteine] was quantitatively measured by high-performance liquid chromatography (HPLC) in the urine of rats given allylamine·HCl (5, 25, 50, 100 and 150 mg kg^{-1}), acrolein (13 mg kg^{-1}), allylalcohol (64 mg kg^{-1}), allylchloride (76 mg kg^{-1}), allylbromide (120 mg kg^{-1}), allylcyanide (115 mg) and cyclophosphamide (160 mg kg^{-1}) by gavage in water. 3-OHPrMCA was measured by HPLC in 24-h urine collections; the lower detection limit was 1.25 μg or 5.6 nmol ml^{-1}. Various doses of allylamine resulted in 3-OHPrMCA excretion at a fairly constant percentage of the dose, ca. 44–48% at 0–24 h and 3% at 24–48 h, indicating rapid metabolism through glutathione conjugation in the first 24h. Similarly, 3-OHPrMCA was recovered in the urine of rats given acrolein (78.5%), allylalcohol (28.3%), allylchloride (21.5%), allylbromide (3.0%), allylcyanide (3.7%) and cyclophosphamide (2.6%). These data indicate that 3-OHPrMCA can be used as a marker of exposure to allylic and other compounds that lead to the metabolic formation of acrolein.

INTRODUCTION

The mercapturic acids (S-subsituted *N*-acetyl-L-cysteine derivatives) are the end-products of an important pathway in the metabolism of xenobiotics, i.e. conjugation to glutathione. This type of conjugation is generally catalyzed by glutathione transferases and has a special importance in the detoxification of reactive compounds.[1–4] There have been a number of reports of 3-hydroxypropylmercapturic acid (3-OHPrMCA) formation following the administration of allylalcohol,[5,6] allylhalides,[7] cyclophosphamide[4,8] and allylamine[9] to experimental animals.

There is no simple method known for the biological monitoring and surveillance of workers exposed to allylamine or related compounds. The ultimate objectives of biological monitoring are to give an estimate of the health risk of an individual exposed to the chemicals and to identify significantly exposed individuals.

Occupational exposure to chemicals can result in disease.[10,11] Medical surveillance by physical examination and, more recently, hematological examinations have been proposed for use in monitoring working populations. Biological monitoring of chemical exposure, through examination of biological fluids such as blood or urine components, is an alternative to address this problem in an occupationally exposed population. Recently, it has been suggested that a reduction in erythrocyte glutathione-*S*-transferase activity could serve as a marker of exposure to acrolein, the aldehyde metabolite of many allylic and related compounds.[12] A few studies[1,13] have examined urinary glutathione conjugation products as potential biological markers of chemical exposure in humans, and have supported the feasibility of this approach.

In the present study we have explored the possibility of measuring 3-OHPrMCA as a biological marker of exposure for chemicals that are metabolized to the toxic aldehyde, acrolein, and are excreted as mercapturic acid in the urine. We conclude that the presence of this mercapturic acid is readily observed as a marker of exposure, and further refinement of the methodology would result in a useful tool in biological monitoring.

MATERIALS AND METHODS

Allylamine was purchased from ICN Pharmaceuticals (Cleveland, OH). Its purity was checked by gas chromatography (GC) and thin-layer chromatography (TLC) and was found to be 98%. Allylamine was given to rats as the HCl salt dissolved in double-distilled water. Acrolein (97%), allylalcohol (99%), allylchloride (98%), allylbromide (98%) and allylcyanide (97%) were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used as delivered. Cyclophosphamide was obtained from the Drug Synthesis and Chemical Branch, National Cancer Institute, Bethesda, MD (kindly donated by Dr Victor J. Ferrans). Solvents used for chromatography were of HPLC grade.

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Chromatographic equipment

High-performance liquid chromatography (HPLC) was performed at 21°C on a Beckman Isocratic liquid chromatograph (Model 330) equipped with an Ultrasphere ODS column (5 μm, 25 cm × 4.6 mm i.d.), and a 3-cm guard column with identical stationary phase. The column effluent was monitored at 210 nm using a Perkin-Elmer Model LC-55 variable-wavelength ultraviolet detector. A 100-μl sample was injected in each case and the flow rate was 1 ml min^{-1}. A data module (Hewlett-Packard Integrator Model 3393A) was used to calculate peak areas and retention times.

Buffer preparation

The mobile phase contained 15 mM potassium dihydrogen phosphate, 10 mM tetrabutylammonium hydrogen sulfate (TBASH) (pH was adjusted to 6.7 with KOH) and acetonitrile (10 : 3, v/v). The mobile phase was filtered through a 2-μm cellulose acetate filter (Millipore, Bedford, MA) and degassed before use.

Animals and treatments

Male Sprague-Dawley rats (220–250 g) (Harlan, Houston, TX) were acclimatized in our Animal Care Facility for 1 week prior to treatment and, following treatment, were housed individually in metabolic cages with free access to drinking water and food (Purina Rat Chow). Five groups (n=3) of rats were treated with allylamine doses of 5, 25, 50, 100 and 150 mg kg^{-1} of body weight by gavage; urine from these rats was collected at 24 and 48 h. Similarly, rats (n=3) were treated with acrolein (13 mg kg^{-1}), allylalcohol (64 mg kg^{-1}), allylchloride (76 mg kg^{-1}), and allylbromide (120 mg kg^{-1}), allylcyanide (115 mg kg^{-1}) and cyclophosphamide (160 mg kg^{-1}) by gavage. These doses were based on published LD_{50} values, except for the dose of acrolein which represents a $\frac{1}{8}LD_{50}$; the acrolein dose was chosen because the LD_{50} resulted in significant mortality at 24 h in a preliminary study. Urine from these rats was collected at 24 h only. Rats treated with water by gavage served as a control.

Synthesis of reference compound

3-OHPrMCA was synthesized and characterized as described earlier.[9]

Isolation, extraction and recovery of mercapturic acid from urine

The total volume of the 24-h urine samples was measured and the sample was centrifuged at 2000 rpm for 10 min. An aliquot of 1 ml of urine was lyophilized. The solid residue obtained was dissolved in 200 μℓ of water and a 100 μℓ sample of each was analyzed by HPLC.

Calibration curve and statistics

From a 1-ml stock solution (conc. 1 mg ml^{-1}) of synthesized mercapturic acid in water, aliquots of 5, 10, 25, 50 and 75 μℓ were added to 1 ml of control urine (concentration range 0.02–0.33 μmol ml^{-1}). The mixture was subsequently processed as described above. A standard curve was constructed by plotting peak areas against known concentrations of mercapturic acid added to control urine; mercapturic acid in unknown samples was calculated from this standard curve. Data are given as the mean result ± SEM. Linear regression analysis was performed on the allylamine dose–response data.

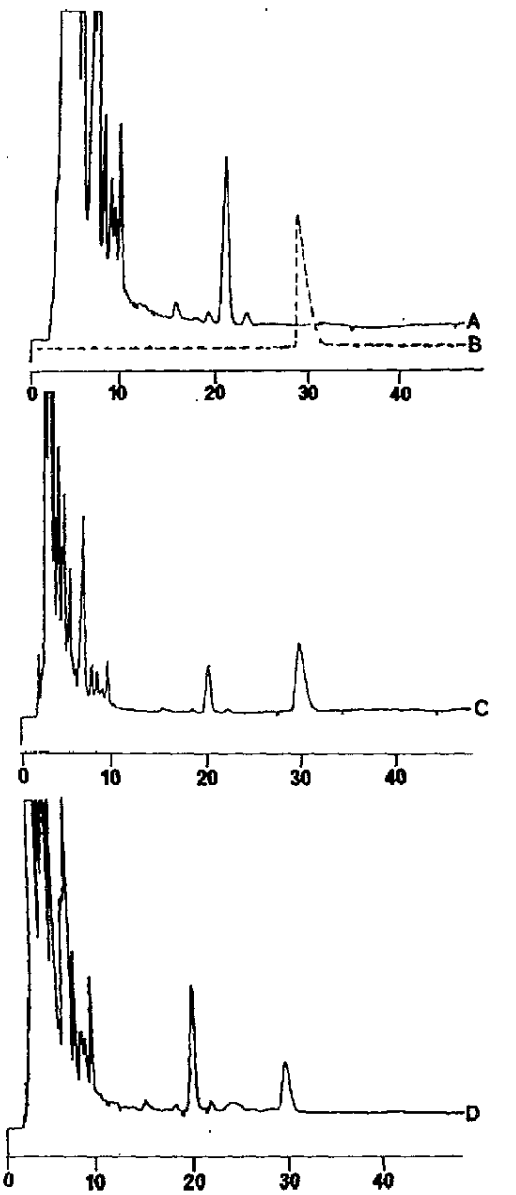

Figure 1. Representative HPLC chromatograms of: (A) control rat urine (24-h collection); (B) synthesized 3-OHPrMCA (dotted line); (C) rat given allylamine (5 mg kg^{-1}, 24-h urine) — rats given all the compounds tested showed identical peaks; (D) control rat urine spiked with 3-OHPrMCA (75 μg ml^{-1}).

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RESULTS

The HPLC method developed for 3-OHPrMCA showed no peak in urine from control rats (29–30 min, Fig. 1A), whereas in control urine spiked with synthesized 3-OHPrMCA a peak was observed at 29 min (Fig. 1D), which corresponded to the peak obtained with a pure sample of 3-OHPrMCA. The standard curve for synthesized mercapturic acid was linear and the lower detection limit was 1.25 μg or 5.6 nmol ml^{-1}. No mortality was observed in any of the treatment groups. Urine from rats treated with each compound tested showed a peak at 29 min (for example, see chromatogram from rat given 5 mg kg^{-1} allylamine, Fig. 1C).

In the dose–response study of 3-OHPrMCA recovered at 24 h in urine from rats given allylamine, the recovery of mercapturic acid was consistent at all doses (44–48% calculated on a molar basis), and a highly significant direct relationship (r = 0.99 by linear regression analysis) was shown between dose and urinary excretion of 3-OHPrMCA (Table 1). In the allylamine-treated rats, the amount of mercapturic acid recovered between 24 and 48 h represented ca. 3% in all groups.

Table 1. Excretion of 3-hydroxylpropylmercapturic acid after different doses of allylamine

Allylamine (mg kg^{-1})	Mercapturic acid recovered[a] (mg)	% Recovery of 3-OHPrMCA at 24 h[b]
5	2.2 ± 0.9	43.8% ± 1.1
25	11.8 ± 0.7	47.5% ± 1.2
50	24.1 ± 0.3	48.3% ± 0.9
100	48.2 ± 0.3	48.2% ± 0.9
150	72.2 ± 0.7	48.1% ± 1.3

[a] The values are means ± SEM (n=3).
[b] Calculated on a molar basis; values are mean ± SD (n=3).

To compare the formation of mercapturic acid in urine samples from rats given different compounds, LD_{50} or $\frac{1}{2}LD_{50}$ doses of acrolein, allylalcohol, allylchloride, allylbromide, allylcyanide and cyclophosphamide were given to the rats. The maximum recovery was observed in urine from acrolein-treated rats (Table 2).

The isolated mercapturic acids obtained from urine of rats given all the test compounds were identified by mass spectral studies. The electron impact mass spectra (EI-MS) of isolated urinary metabolites from rats given allylamine, acrolein, allylalcohol, allylchloride, allylbromide, allylcyanide and cyclophosphamide were identical to that previously reported for 3-OHPrMCA.[9]

DISCUSSION

The present study demonstrates the consistent excretion of 3-OHPrMCA in the urine of rats treated with allylic or related compounds known to result in the formation of acrolein as a metabolite. Most of the 3-OHPrMCA was excreted during the first 24 h. In dose dependency experiments with allylamine (given 5–150 mg kg^{-1}), a remarkably direct relationship was shown, even at low doses of toxin. In rats treated with acrolein, the maximum formation of mercapturic acid was also obtained in urine within 24 h. The percentage of acrolein excreted as 3-OHPrMCA was the highest of all the compounds tested.

These studies, therefore, clearly support the concept that these allylic compounds are metabolized to acrolein.[9] Such metabolism could occur via allylalcohol or through amine oxidation.[14] The lower percentage of dose recovered in the urine after cyclophosphamide administration supports previous studies – indicating that there is no direct conversion of cyclophosphamide to acrolein but rather metabolism occurs via aldophosphamide, followed by spontaneous formation of phosphamide mustard and formation of acrolein.

3-OHPrMCA is easily detectable by this method at low levels in rat urine. The rat has been shown to be a good model for man with respect to glutathione conjugating ability.[15] Since 3-OHPrMCA has been

Table 2. 3-Hydroxylpropylmercapturic acid excretion[a] after administration of test compounds

Compound	$\frac{1}{2}LD_{50}$ (mg kg^{-1})	Mercapturic acid excreted (mg/24 h)	Recovery of 3-OHPrMCA[b] (%)
Allylamine	150	72.3 ± 0.7	48.7 ± 1.3
Acrolein	13[c]	10.1 ± 1.3	78.5 ± 2.2
Allylalcohol	64	17.3 ± 0.7	28.3 ± 0.9
Allylchloride	76	11.9 ± 0.7	21.5 ± 0.3
Allylbromide	120	1.6 ± 0.5	3.0 ± 0.7
Allylcyanide	115	3.6 ± 0.3	3.7 ± 0.9
Cyclophosphamide	160	0.9 ± 0.2	2.6 ± 0.3

[a] In 24-h urine collection.
[b] Calculated on a molar basis; values are mean ± SEM (n=4).
[c] LD_{50}.

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determined in these experiments to be an important urinary metabolite of allyl compounds, this method — properly applied and refined for measurement of this metabolite — should prove to be a valuable tool in monitoring human exposure to these important industrial chemicals.

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